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AN INTRACELLULAR STUDY OF SYNAPTIC POTENTIALS  
DURING VOLUNTARY MOVEMENT IN PRIMATE MOTOR CORTEX

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AN INTRACELLULAR STUDY OF SYNAPTIC  
POTENTIALS DURING VOLUNTARY MOVEMENT  
IN PRIMATE MOTOR CORTEX

by

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Running Head:

Intracellular PSPs of Primate Motor  
Cortex during Voluntary Movement

## INTRODUCTION

Recent studies on the motor cortex neurons in the unanesthetized behaving monkeys have revealed that activities of the pyramidal tract neurons ( PTNs ) are related to an execution of voluntary movement <sup>10, 11, 12, 15, 16</sup>. PTN discharge rate is linearly related to the weight of load against the voluntary contracting muscle or to a force produced under isometric contraction <sup>11, 29</sup>. In a task of simple reaction with visual cue, reaction-related PTNs are activated 100 ms after the visual cue and 50 - 100 ms before the movement onset <sup>10</sup>

As for the EEG studies related to the voluntary movement, there are several reports. Very small DC potential, related to the movement onset, is detected by averaging technique <sup>5, 6, 34, 35</sup>. This potential has several components designated as readiness potential ( RP ), premotion positivity ( PMP ), and motor potential ( MP ). According to Deecke et al. <sup>5, 6</sup> before a self-paced voluntary movement, such as finger flexion, MP appeared 50 - 80 ms prior to the movement. This potential is observed in a localized precentral area contralateral to the contracting muscles. Vaughan et al. also found localized potentials <sup>34, 35</sup>. MP may be considered as an aggregate PTN activities before the movement <sup>5, 6</sup>. In addition to this MP, a slowly developing negative potential ( RP ) and positive potential ( PMP ) appeared 700 - 1000 ms and 80 - 120 ms before the movement, respectively. As these earlier components were observed in both hemispheres and larger in other areas such as parietal region, hence, considered as not directly related to motor cortex activity. Such potentials are not studied well in simple reaction task situation. But in CNV studies, alarming stimulus, visual or auditory, induced

simple reaction. From these studies, it might be clear that before the onset of the movement, there are potentials related to synaptic and spike activities of PTNs. However, exact timing relations are hard to be inferred from the EEG surface recordings.

To understand synaptic mechanisms responsible for PTN activation associated to the movement, extracellular single unit studies and EEG studies would not give crucial answers. Intracellular recordings of neurons, related to voluntary movement, seems to be necessary. These questions such as, when synaptic inputs arrive at the motor cortex neurons, how synaptic inputs drive the PTN, or physiological characteristics of the synaptic potentials, will be answered by intracellular recordings from behaving monkeys. However, it appears technically infeasible to keep the electrode inside the neuron of awake behaving preparation in a stabilized state for enough time to be investigated. Such studies in chronic monkeys are not yet available. Intracellular techniques had to be developed before attempting to record movement-related synaptic potentials. Only several reports are available: Yokota et al.<sup>39</sup> recorded hippocampal neurons of squirrel monkeys and obtained olfactory evoked synaptic potentials, Skrevitzky et al.<sup>28</sup> recorded visual cortex neurons of rabbits and investigated the effect of reticular suppression, and Woody et al.<sup>38</sup> recorded auditory cortex neurons of cats and investigated plastic changes of neuronal excitability in classical conditioning. In the motor cortex there is only one report. Woody et al.<sup>37</sup> measured excitability of coronal-pericruciate neurons of the cats in the course of acquisition of the eye blink conditioning.

In the present study, chronic intracellular techniques, similar to those of Woody et al.<sup>37</sup>, were applied to the motor cortex of the rhesus

monkeys, and timings of synaptic potentials associated with movements were studied. Electrical membrane properties were also studied. Preliminary report was presented in the 54th General Meeting of the Physiological Society of Japan <sup>23</sup>

## METHODS

Five rhesus monkeys ( Macaca mulatta, 3.5 - 7.9 Kg ) were used for the chronic intracellular studies. Recordings were made from the motor cortex, while they were performing a visually-guided voluntary movement. After the chronic experiments were ended, three of them were used for acute experiments in anesthetized condition. Additionally in two cats intracellular potentials were recorded from the PTNs in anesthetized state.

### Training and paradigm

All monkeys were trained to perform visually-guided wrist movement task. Usually they were housed in individual cages in a cage room and brought to a primate chair whenever training or recording was performed. Monkeys sit willingly on the chair, and accepted the head fixations. Throughout the experiments they were cooperative. Manipulandum was a vertical grip handle ( Fig.1A ). The monkey in the chair held the vertical handle and rotated it by flexing or extending the wrist for about 30° between two stop zones. Each zone consisted of 10° angle width. Maintaining the handle for 3 s within one of stop zones, a visual cue ( red LED; Sharp Co., type GL-31AR ) was presented to the monkey. It was about 30 cm in front at face level. As soon as the cue was presented, monkey flexed or extended the wrist until the gripped handle reached opposite stop zone within 400 ms. Then a drop of reward juice was guided to the mouth. Tone pip ( 1 KHz, 1 s duration ) was simultaneously emitted, and the cue lamp was turned off.

Fig.1

After 3 - 10 s inter-trial interval time ( ITI ), next trial started, then the monkey rotated the handle to another stop zone. The monkey usually performed 300 - 700 trials in a day. Training was continued till the monkey performed the task securely within 200 - 350 ms reaction times. It took 25 - 40 days for monkeys to perform the task. During an intracellular recording the tone pip was emitted everytime when the target zone was hit, and reward juice was given in every two to five successful trials ( fixed ratio task ) Displacement of the handle was measured by a voltage change of coaxially attached potentiometer ( Copal Co., type J45S, 10 K $\Omega$  ). as shown in Fig.1A.

### Surgery

After an acquisition of the task a preliminary surgery was performed under Nembutal anesthesia. Conventional concentric bipolar stainless steel electrode was implanted into the pontine pyramid ( AP = 0, L = 1, H = -10 to -12 ) Pt-Ir wire or silver ball electrode ( or occasionally stainless bolt ) was used as an indifferent electrode for the intracellular recording, and was implanted on the surface of the dura mater at the frontal pole of the midline. DC potential drift caused by this indifferent silver or stainless electrode was less than 5 mV / 20 min. and, therefore, was not considered so serious in this experiment. As illustrated in Fig.1A, two head holder pipes of 8 mm diameter and four stainless bolts were implanted and fixed by dental cement. Head holder pipes were electrically isolated from the monkey. The skull, covering the motor area, was widely exposed and kept dry.



### Recording apparatus

For intracellular application relatively heavy and tightly fixable primate chair was made to avoid artifacts due to behavioral and postural changes of the monkey ( Fig.1A ). A stereotaxic frame ( Takahashi Co., Noken type ) was mounted on the top of the chair so that the head of the monkey could be rigidly fixed by two thick parallel bars<sup>24</sup>, as shown. The monkey was electrically isolated from the chair, and the handle was mechanically isolated from the chair.

The glass micro-pipettes were prepared by a vertical puller ( Takahashi Co., Type I ) from Pyrex glass capillary tubes of 1 mm outer diam. which contain 0.1 mm diam. micro-filament inside ( Takahashi Co. ). Micro-pipettes were manually filled with 2 M K-citrate or 2 M NaCl just before the use. Resistances were 12 - 30 M $\Omega$ .

Micro-pipette was attached to the top of a hydraulic micro-manipulator ( Narishige, type MO-8 ). Guide tube was also attached to the manipulator. Both electrode and guide tube could be moved independently or in combination. The guide tube tip was sharpened to have two peaks at opposite positions, as illustrated in Fig.1B. Intracellular potential was led out via Pt-Ir wire to a pre-amplifier designed to inject constant current<sup>25</sup>, and monitored by an oscilloscope ( Tektronix, 7403N ).

### Recording

Previous to recordings, a small bur hole ( 2.5 mm diam. ) was drilled in the skull over the hand area of the motor cortex. Dura was

carefully and gently incised for 2 mm with a fine needle under binocular microscopic vision. The tip of the guide tube slightly pressed the dura, and the dura was opened only between two peaks of the guide tube. Electrode was deepened into an exposed cortex through an opened dura, as shown in Fig.1B. All penetrations were perpendicular to a stereotaxic horizontal plane. Through one drilled bur hole, for three to four days neuronal activities could be recorded via penetrations. Seven to nine holes were made in a single hemisphere. The electrode was advanced gradually by passing 0.1 ms pulse at 100  $\mu$ A intensity, monitoring antidromically generated field potential, while the monkey was performing the task. Just after a successful intracellular penetration, DC low gain potential ( DC - 10 KHz ), AC high gain potential ( time constant; 0.1 s ), displacement of the handle and onset timing of the visual cue were recorded in 4-channel FM magnetic tape recorder ( TEAC, R-400, speed 30 in./ s ) Within 30 s after an intracellular penetration PT stimulation and measurement of resistance were quickly finished.

At the end of the experiment bur hole was filled with the bone wax.

#### Acute experiments

In 3 monkeys and 2 cats, acute experiments were performed to confirm that membrane properties or spike time course were not different from those of the unanesthetized, and to compare membrane properties between two species. Before sacrificing the chronic monkeys, they were anesthetized by Nembutal ( 35 - 40 mg / Kg, i.p. ). Addi-

tional dose of Nembutal was given intravenously every 2 hours during the experiment. The preparation was artificially respired ( 15 - 20 / min. ), and rectum temperature was maintained between 36 - 38.5°C. Cerebro-spinal fluid was drained through cisterna magna to reduce movement artifacts. Skull over the motor area was opened and dura was incised. In cats PT electrode was implanted stereotaxically at AP = 0, L = 1, H = -12. Recording electrode and other procedures were the same as those in chronic experiment. When the experiment was over, the preparation was deeply anesthetized by Nembutal, and perfused by saline followed by 10 % formaline.

#### Recorded sites

Fig.2 By visualizing the formaline-fixed brain, holes were easily detected where connective tissues developed. Fig.2 illustrates schematically the penetrated points of all monkeys at the cortical surface in which successful intracellular recordings were obtained. Results of the right hemisphere were transformed upon the left side. Occasionally, more than three neurons could be recorded in a single penetration. As illustrated, most of recorded sites were within the upper limb area, including the shoulder area.

## RESULTS

### General

A glass micro-pipette was deepened step by step into the motor cortex, while the monkey was performing a visually-guided wrist movement. Sixty-three neurons were recorded intracellularly, and 22 were identified as PTNs in which the pyramidal tract stimulation ( 100  $\mu$ A ) evoked antidromic spike potential without preceding prepotentials. They had latencies of 0.6 - 1.9 ms. Before the penetration a negative spike was superposed upon antidromically evoked field potential ( more than 200  $\mu$ V positive peak with about 0.7 ms latency ) Immediately after the penetration injury spike discharges lasted for 10 to 20 s. Criteria of intracellular recordings of these neurons were 1) the presence of an abrupt negative potential shift of more than 30 mV immediately after the penetration, 2) a negative potential shift lasting for at least 30 s, and 3) an ability to generate positive spike potentials trans-synaptically, superposing upon the negative potential shift.

FiFiig.3

An episode of a PTN penetration is illustrated in Fig.3. A micro-pipette filled with K-citrate was gradually deepened into the cortex while the monkey was performing the task. As soon as an abrupt DC potential shift of about 60 mV occurred and simultaneously injury discharges occurred, tape recording was started ( B ). Fifteen s later injury discharges disappeared ( C ), and spike bursts were observed transiently during flexion ( upward deflexion of lower trace ) and extension movements ( downward deflexion ) Bursts appeared immediately after visual cue onset, as shown by arrows of lower traces. Pyramidal

tract stimulation evoked a typical antidromic spike ( A ) There was an overshoot of several mV, and after-hyperpolarization potential, as observed in other vertebrate PTNs<sup>26, 27, 31</sup> After a stabilized recording for 4 min., spikes and synaptic potentials were not evoked any more. During this period negative potential decreased gradually to -20 mV. If recording electrode was withdrawn from inside the neuron, DC level returned to an extracellular baseline of 0 V. and no remarkable extracellular potential was recorded during movement ( D ).

#### Synaptic potentials preceding wrist movement

When the monkey was waiting for a coming visual cue, intracellular membrane potential was never steady and fluctuated irregularly with or without occasional spikes for up to 8 mV. Examples are seen in Fig.3C. These were considered as aggregates of various EPSPs and/or IPSPs. This so called " background noise " was clearly observed in 14 neurons, including 8 PTNs, which had task-related spike activities. In these, visual cue induced the depolarization with or without spikes before the displacement was started. Since corresponding extracellular potentials were not clear if the electrode was outside of the neuron, these depolarizations are considered as excitatory post-synaptic potentials, and hereafter, these will be designated as ( depolarizing ) PSPs. The values of critical depolarization for spikes were 2 - 6 mV in 14 neurons.

Fig.4      Fig.4A and B illustrate examples of membrane potential changes during voluntary movement. Irregular potential changes of small sizes were always present. In A, as soon as visual cue was presented, PSP

appeared, multiple spikes superposed, and handle was rotated by flexion movement. PSP onset was indicated by small arrows below the membrane potential traces. Its onset latency was 100 ms, and then the first spike appeared 60 ms later. It was 90 ms earlier than onset of handle displacement. Discharge interval was the shortest just before the movement onset where the membrane potential was most depolarized ( 10 mV ). The depolarization of 5 - 6 mV was necessary for the spike initiation. Even when the movement was finished, PSP was still depolarized and subsided gradually. In B, extension movement was started after the visual cue. PSP appeared with a similar latency of 100 ms, as indicated by small arrows. Five spikes superposed transiently before the movement onset. PSP returned to the pre-trial level during the movement was still on. Spike latency was 180 ms and later than that in A. Duration and amplitude of the PSP were larger in flexion than in extension trial.

Fig.5 Another examples of intracellular PSPs are illustrated in Fig.5 from a non-PTN. Similar to Fig.4, PSPs were produced in either phase of the wrist movement with 120 - 150 ms latency after visual cue. PSPs were larger in extension than in flexion, and occasionally in flexion trial no PSPs were observed. Spike activities appeared before extension (  $E_1$ ,  $E_2$ ,  $E_3$  ), and were not observed in flexion (  $F_1$ ,  $F_2$  ). If the electrode was moved outside the neuron, no potential changes in association with intracellular depolarization were seen (  $E_0$  ). Hyperpolarizing potentials, expected to contribute to the reciprocal antagonistic movements, were not observed in 14 investigated neurons.

Fig.6 Onset timings of the PSPs were studied, in selected neurons, by averaging, taking different time events as zero times. In Fig.6, from a non-PTN, shown in Fig.5, spike trains of seven extension trials were



illustrated at top, its histogram in middle, and averaged PSPs in bottom. In A, these were aligned at visual cue onset ( L ) PSPs started 80 ms after cue onset, as shown by an arrow, and rise gradually. Spike appeared 80 ms later. In B, events were aligned at onset of handle displacement ( H ) An abrupt rise of PSPs occurred about 20 ms before the first spike. PSP onset was recognized earlier than abrupt rise. In C, the response was aligned at onset of the first spike activity after visual cue ( S ). PSPs preceded the first spike by 40 ms. In this neuron about 6 mV depolarization was necessary for spike generation. Time courses of averaged PSPs during the periods when depolarization exceeded a critical firing threshold value of 6 mV ( upper parts of the horizontal broken lines in A, B, and C ) were comparable to those of spike activities shown as histograms in A, B, and c, respectively. Those results would show a correlation between depolarization amplitude and spike activities. If PSPs during flexion movement of this neuron were averaged, depolarization of about 3 mV started 180 ms after cue onset and continued for 300 ms while spike activities were scarcely seen during wrist movement ( not illustrated ).

Degree of variability of PSP latencies of various timing relations, and time differences from PSP onset till movement onset were studied in selected neurons. Table I shows measurements from two neurons, that is, from a PTN and a non-PTN. Means and SDs of onset timing of PSPs and spikes with respect to visual cue onset or movement onset were calculated in 11 flexion trials in a PTN ( A ), and 7 extension trials in a non-PTN ( B ). Reaction time ( 1 ), PSP latency ( 2 ), spike latency ( 3 ), a time from PSP onset to spike ( 4 ), a time from PSP onset to handle displacement ( 5 ), and a time from the first spike to

handle displacement ( 6 ) are shown. Both in A and B, SD values of ( 6 ) are relatively small and about one-half of values of ( 1 ) - ( 5 ). Therefore, it may be said that spike activities are " time-locked " to the movement, rather than visual cue or PSPs are. This tendency was also recognized in other 5 neurons. In these two neurons it is said that the PSP onsets were not so well time-locked neither to visual cue nor the movement, because the SD values of ( 2 ) - ( 5 ) were not smaller than that of ( 6 ) of respective neurons. Differences of the PSP onset latencies between A and B were not detected.

In general, differences of the PSP onset latencies between PTNs and non-PTNs were not detected in examined neurons. It is to be emphasized that relatively long time was necessary until PSP reached to a critical firing levels, and those depolarizations had slow rise times.

As known in some synapses, the PSP reduces spike potential amplitudes by 13 mV ( EPP )<sup>14</sup>, or 5 - 8 mV ( frog motoneuron )<sup>13</sup>, and this, called " short circuit action ", is explained as due to a conductance change of the membrane<sup>13, 14, 21</sup>. Such effect was observed in only one neuron out of 14 neurons. When movement-related PSPs were elicited, both spike and after-hyperpolarization potentials decreased in size, though the decrement was not so great. An example of " short circuit

Fig.7 action " during PSP, related to voluntary movement, is shown in Fig.7.

Before the visual cue, this neuron showed injury discharges with a frequency of about 50 Hz. During the highest period of spike activities after-hyperpolarizations disappeared, and spike height decreased by about 3 mV ( 4 % ). Spike activities increased 170 ms before the flexion movement. Earliest sign of amplitude decrements of after-hyperpolarizations occurred almost simultaneously with spike discharges.

Those reductions are thought to be due to conductance increase by excitatory synaptic mechanisms. In other neurons the " short circuit action " was not so clear as this.

### Static electrical properties of neurons

Fig.8 In 46 neurons, including 18 PTNs, membrane resistances were measured. Resistance was calculated from an IR drop across the membrane produced by passing a hyperpolarizing current of  $1.2 \times 10^{-9}$  A for 50 ms duration through a recording micro-pipette. In each neuron, obtained values were the average of five responses. Relations between resting potentials and membrane resistances of measured neurons were shown in Fig.8. Frequency histogram of membrane resistance of neurons is shown in Fig.8B. Mean resistances of PTNs and non-PTNs were  $3.5 \pm 1.7 \text{ M}\Omega$  and  $4.5 \pm 2.5 \text{ M}\Omega$ , respectively. These values were slightly lower than those obtained from coronal-pericruciate neurons in awake cat<sup>37</sup> Fig.8C illustrates the frequency histogram of membrane potentials of 46 neurons, and Fig.8D illustrates that of whole sample neurons. Mean potential was  $58 \pm 12 \text{ mV}$  in 22 PTNs and  $54 \pm 13 \text{ mV}$  in 41 non-PTNs. Correlations of membrane potentials ( abscissa ) vs membrane resistances ( ordinate ) were plotted in Fig.8A. Points distributed widely and evenly. As seen, no correlations were found between membrane potential and resistance values in both PTNs and non-PTNs groups (  $r = -0.04$  in PTNs and  $r = -0.05$  in non-PTNs ). As obtained resistance values were not affected by their membrane potentials, a possibility of current leak caused by damaging the penetrated membrane might be non-significant Therefore, it can be said that these resistance values were comparable to those reported in acute preparations<sup>26</sup>

Relations between latencies of antidromic spike of PTNs and resistances were studied to obtain an insight on relationship between neuron sizes and conduction velocities of their axon. In Fig.9, there is a clear correlation between antidromic latency ( abscissa ) and resistance ( ordinate ) (  $r = 0.66$ ,  $n = 16$  ). The shorter the latency, the lower the resistance. Slope of regression line was comparable to the results of fast-PTNs in the anesthetized cat <sup>26</sup> It may be said that these relations are common in cat and monkey PTNs.

The time course of the potential change by a hyperpolarizing current pulse was investigated in awake and anesthetized monkeys. Fig.10 shows examples of PTNs in anesthetized monkey ( A - E ), and also in anesthetized cat ( F H ). The PTN of a monkey had 1.0 ms antidromic latency and 60 mV resting potential ( A ) Upper trace of A shows depolarizing current monitoring of 1.0 ms, 0.5 nA for the calibration. When the constant hyperpolarizing current pulses were delivered across the membrane, almost stepwise shift of membrane potential was observed in monkeys ( C - E ) The earliest portion of the potential shift was shown in B. Within 2 or 3 ms its decay shift was almost finished. Such short time decays were observed in all of 10 measured neurons. When normalized time decay of the membrane potential was plotted in semi-logarithmic graph ( B' ), it was revealed that time course could be approximated by two curves with different time constants, as described previously <sup>33</sup>. Though two different time constants were recognized, the values (  $\tau_0 = 0.45$  ms,  $\tau_1 = 0.10$  ms in 10 neurons ) were far smaller than those reported in PTNs, motoneurons, giant cells of red nucleus in anesthetized cats <sup>3, 17, 26, 33</sup> The time constant values did not depend on the amplitude of the recorded membrane potential, whether the

penetration was in awake or not, whether the recorded neuron was a PTN or a non-PTN.

In two cats PTN activities were examined by the same recording system, as a control experiment ( F - H ). Time courses of the membrane potential shift by a hyperpolarizing current, as previously reported, were invariably slower than in monkeys. Moreover, overshoot component could be observed in the cats, as reported <sup>3, 17, 26</sup>. Therefore, fast time decay observed in monkeys was a unique property of the neurons in monkeys. A possibility that current leak occurred through damaged membrane, caused by micro-pipette penetration, could be excluded because, even in neurons with high resistance values, time decays were still fast. If the penetration site of the recording micro-pipette was in dendrite, cable property or capacitance property would be different from those in soma. But it is hardly considerable that all recording sites were within dendrites. Although it may be expected that the capacitance properties of the membrane of neurons in monkeys would be quite different with respect to passive charge shift, exact reasons could not be determined in this experiment.

## DISCUSSION

While unanesthetized monkeys were performing a visually-guided wrist movement, intracellular responses, related to an initiation of voluntary movement, were recorded from the upper limb area of the motor cortex. Intracellular potentials of more than 30 mV were recorded from 63 motor cortex neurons. It may be argued that, as a criterion of intracellular responses, negative potential shift of 30 mV is low, compared with a criterion by other authors on the acute experiment<sup>31, 32</sup>. But spike activities of more than 20 mV were always superposed upon the post-synaptic potentials, and furthermore, according to Kelly and Van Essen<sup>20</sup>, an intracellular dye injection is possible, even when the membrane potential was less than 20 mV. Therefore, this value is taken as a criterion of intracellular membrane potential in this paper, though the recorded potentials may not be from so called "good" neurons.

Out of 63 neurons, 22 were identified as PTNs ( 35 % ). The remainings were designated as non-PTNs in this paper. Among these non-PTNs, probably PTNs were included, because in most of recorded neurons stimulus current intensity for identifying was arbitrarily limited to less than 100  $\mu$ A, in order to prevent possible damages to the neurons due to excessive movements, and this intensity was about one-third of the supramaximal for the antidromic field potential.

All PTNs except for one ( antidromic latency; 1.9 ms ) were considered as rapidly conducting PTNs<sup>9, 31</sup>. Reasons why only one slowly conducting PTN is sampled is probably due to facts that, firstly, smaller size neurons are hard to be penetrated by a micro-pipette, secondly, superficial layers of the cortex where small PTNs are abundant ( layer



II - III ) might have been destroyed by pressure of the guide tube.  
Most of successful recordings were obtained from deeper layers.

#### Slow depolarization associated with voluntary movement

In both PTNs and non-PTNs depolarizing PSPs related to the voluntary movement appeared 70 - 180 ms prior to onset of the movement, as estimated by handle displacement, and were 80 - 180 ms after the visual cue. Intracellular spike activities preceded the movement by 40 - 100 ms, though some were activated as earlier as up to 150 ms. These were comparable values to extracellular PTN activities, as was reported<sup>10</sup>. There were about 20 - 80 ms for PTNs from their onset to cause spike activities. These values are relatively long, Compared with the rise time of specific EPSPs evoked by electrical stimulation such as ventro-basal thalamic input to PTNs<sup>32</sup>, or Group Ia input to motoneurons<sup>8</sup>. In addition, gradient of rise was different in different neurons. From these varied onset and irregular time courses, it is likely that those PSPs are resultant of a summation of small EPSPs.

Inhibitory synaptic inputs to the motor cortex neurons have been physiologically demonstrated<sup>2, 22</sup>, and hyperpolarizing potentials were expected to participate in inhibitory mechanisms during reciprocally controlled antagonistic movement, but clear IPSPs during voluntary movement were not seen in this survey. It may be probable that inhibitory mechanisms may not play essential roles in the task performance of relatively slow and smooth movement, as chosen in this experiment. And an other possibility can not be excluded that PSPs, recorded in this experiment, represented the general effect due to other muscle movements

which were activated non-reciprocally. But this result is not conclusive because sampled numbers of neurons are small.

### Sources of synaptic inputs

Anatomical and physiological studies show that the motor cortex receives synaptic inputs from various regions, such as area 3, 6, and supplementary motor area for direct cortico-cortical connections<sup>7, 18, 19</sup>, specific and non-specific thalamic nuclei and basal ganglia<sup>1, 4, 30, 36</sup>. In the current experiments no attempts were made to determine sources of inputs of PTNs, related to visually-guided voluntary movement. In view of facts of variable PSP latency and slow development, it is likely that inputs are activated non-synchronously, and less likely that such PSPs are evoked only via a specific pathway, such as thalamo-cortical pathway. As reported in cat PTN, EPSPs caused by thalamic VB stimulation had a sharp rising phase with a latency of 2.5 ms<sup>32</sup>. The movement-related PSPs might contain synaptic potentials due to indirect activations by interneurons of the motor cortex which evoke EPSPs primarily at the soma region and/or due to direct activations at the dendritic region away from the soma. Presence of a weak "short circuit action" upon spike height may support a notion that PSPs of dendritic and soma origin are not exclusively present, it is more likely that those two are compatibly present. It appears likely that at least dendritically evoked PSP may be included in the production of the voluntary movement.

## SUMMARY

Intracellular recording technique was applied to the precentral motor cortex of the unanesthetized, chronically behaving rhesus monkey, sitting in a primate chair. It was attempted to record PSPs responsible for an initiation of the voluntary movement.

In total, 22 pyramidal tract neurons ( PTNs ) and 41 non-pyramidal tract neurons ( non-PTNs ) were successfully penetrated in 5 monkeys, while the monkey was performing a flexion-extension wrist movement after a visual cue ( reaction time; 200 - 350 ms ) They showed negative membrane potential shift of at least 30 mV for more than 30 s.

Slowly rising PSP appeared 80 - 180 ms after the visual cue, and was 70 - 180 ms prior to an onset of the movement. Spike activities superposed upon this slow PSP with 20 - 80 ms rise time and more than 2 - 6 mV depolarization ( 8 PTNs and 6 non-PTNs ) Since these depolarizations were variable in magnitude and latency, these were considered to be summated small EPSPs.

Membrane resistance was measured from an IR drop by a hyperpolarizing current (  $1.2 \times 10^{-9}$  A ) passed through a recording electrode, and yielded values were  $3.5 \pm 1.7$  M $\Omega$  in 18 PTNs and  $4.5 \pm 2.5$  M $\Omega$  in 28 non-PTNs. There was a linear relationship in PTNs between membrane resistance and antidromic latency from pontine pyramid.

Because of the time course of PSPs, their possible dendritic origins were discussed.

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## LEGENDS FOR FIGURES

Fig.1

A monkey in a primate chair for intracellular recording, and a system for micro-pipette penetration. A: a drawing of a heavy-duty primate chair, to which the monkey's head was rigidly fixed by two parallel bars of 8 mm diam. to stereotaxic frames of both sides at the top. The monkey was holding a vertical handle by his left hand. A hydraulic micro-drive could be attached to the frame stereotaxically ( H; handle, P; potentiometer ) B: a drawing of a recording micro-pipette with its guide tube attached to the top of the micro-drive via guide holder. During penetration, incised dura was slightly opened and pressed by two peaks of the guide tube.

Fig.2

Locations of the micro-pipettes penetrations. On the cortical surfaces of five monkeys, points of successful intracellular penetration are shown. Circle size corresponds to number of recorded neurons in a single track. Large circle indicates that more than three neurons were recorded in a single penetration, middle one two neurons, and small one one neuron. Numbers above each drawing indicate total number of penetrations of each monkey. ( c.s.; central sulcus, a.s.; arcuate sulcus, p.s.; principal sulcus, s.c.s.; supra-precentral sulcus )

Fig.3

Intracellular recordings of a PTN during wrist movement. A: antidromic spike potential of 0.8 ms after pontine pyramid stimulation ( 0.1 ms, 100  $\mu$ A, 2 Hz ). Five sweeps were superposed. B: recording of injury discharges 10 s after a penetration, obtained while the monkey was performing the wrist movement. C: recording one minute after penetration. D: extracellular potential near the penetrated neuron. Membrane potential was about 68 mV. Lower traces of B, C, and D represent angular displacement of the handle. Upward movement shows wrist flexion and downward extension. Onset timings of visual cue are indicated by vertical arrows.

Fig.4

Intracellular responses of a PTN during wrist movement. From the same PTN, illustrated in Fig.3. Spike activities in flexion movement ( A, FL ) is greater than in extension ( B, EXT ). Smaller arrows indicate PSP onset. AC; AC high gain recording of the intracellular response, DC; its DC low gain recording, H; displacement trace.

Fig.5

Intracellular responses of a non-PTN during wrist movement. Three trials of extension (  $E_1$ ,  $E_2$ ,  $E_3$  ) and two trials of flexion (  $F_1$ ,  $F_2$  ) are shown. Traces of  $E_0$  shows extracellular response. DC high gain recording ( upper ), DC low gain recording ( middle ), and handle displacement ( lower ) are shown in each trial.

Fig.6

Averaged PSPs ( lower ) and spike activities ( upper and middle ) from extension trials in a neuron illustrated in Fig.5. A: dot displays of spikes, taking the visual cue onset as zero axis ( L ). Filled triangular points indicate the movement onset. Arrow indicates a PSP onset. B: responses were aligned to the onset of handle movement ( H ). C: responses were aligned to the first spikes of each trial ( S ).

Fig.7

Spike potential reduction during flexion movement-coupled PSP, from a PTN described in Table IA. Two traces of DC high gain recording ( upper ), DC low gain ( middle ), and handle displacement ( lower )

Fig.8


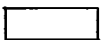
Relations between membrane resistance ( ordinate ) and membrane potential ( abscissa ) of 18 PTNs and 28 non-PTNs. A: resting membrane potential-resistance relations. Open circles indicate PTNs, and filled ones non-PTNs. B: frequency distributions of membrane resistance values of PTNs (  ) and non-PTNs (  ) sampled in A. Ordinate; resistance in M $\Omega$ . C: frequency distributions of membrane potentials sampled in A. D: membrane potential histogram of total sampled neurons ( N = 63 ).



Fig.9

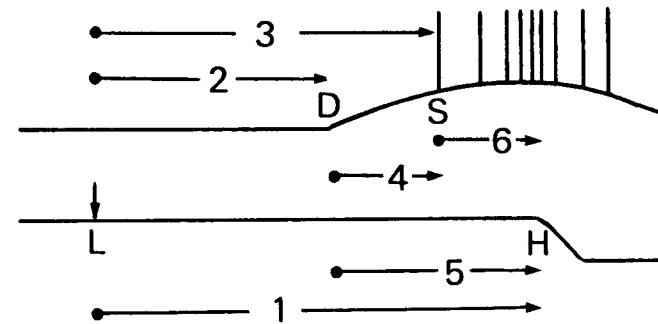
Antidromic latency-membrane resistance relations of 16 PTNs.  
Ordinate; membrane resistances in  $M\Omega$ . Abscissa; antidromic latencies  
in ms.

Fig.10

Membrane potential changes of PTNs to constant hyperpolarizing  
current pulses in a monkey PTN ( A - E ), and in a cat PTN ( F - H ),  
in Nembutal-anesthetized state. Upper traces indicate current inten-  
sities and lower traces membrane potentials. A: antidromic spike with  
1.0 ms latency and 60 mV resting potential, and depolarizing current  
for calibration. B: earlier part of the hyperpolarization when current  
pulse was injected. B': semi-logarithmic plots of time course of the  
potential shift of B. C - E: hyperpolarizations in different current  
steps. F - H: hyperpolarizations in different current steps in the  
cat.

Table I

Timing relations of  
depolarizing PSPs and spikes



	1	2	3	4	5	6
A	349 $\pm$ 31	169 $\pm$ 32	207 $\pm$ 33	39 $\pm$ 29	181 $\pm$ 35	142 $\pm$ 12
B	280 $\pm$ 56	147 $\pm$ 71	225 $\pm$ 61	77 $\pm$ 63	133 $\pm$ 75	55 $\pm$ 37
( mean $\pm$ SD ) ms						

Means and SDs of the time relations illustrated above are calculated from eleven extension trials in a PTN ( A ) and seven flexion trials in a non-PTN ( B ). Letters in the insert indicate event timings. L; visual cue onset, D; depolarizing PSP onset, S; spike onset, H; handle displacement onset. L-H; reaction time ( 1 ), L-D; latency of depolarizing PSP ( 2 ), L-S; latency of the first spike ( 3 ), D-S; PSP time to spike initiation ( 4 ), D-H; PSP time to the displacement onset ( 5 ), S-H; spike time to the displacement onset ( 6 )

Fig.1

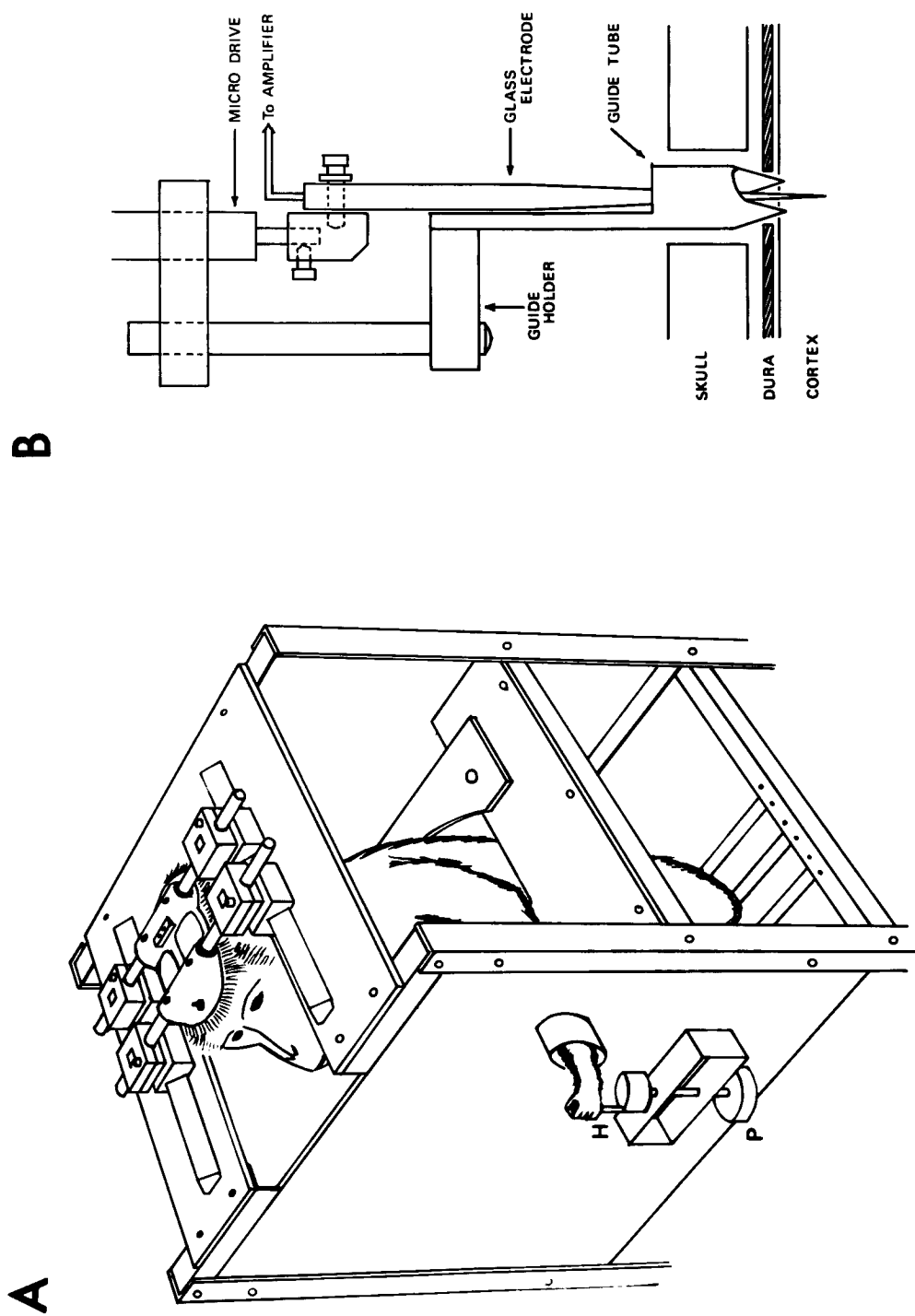


Fig. 2

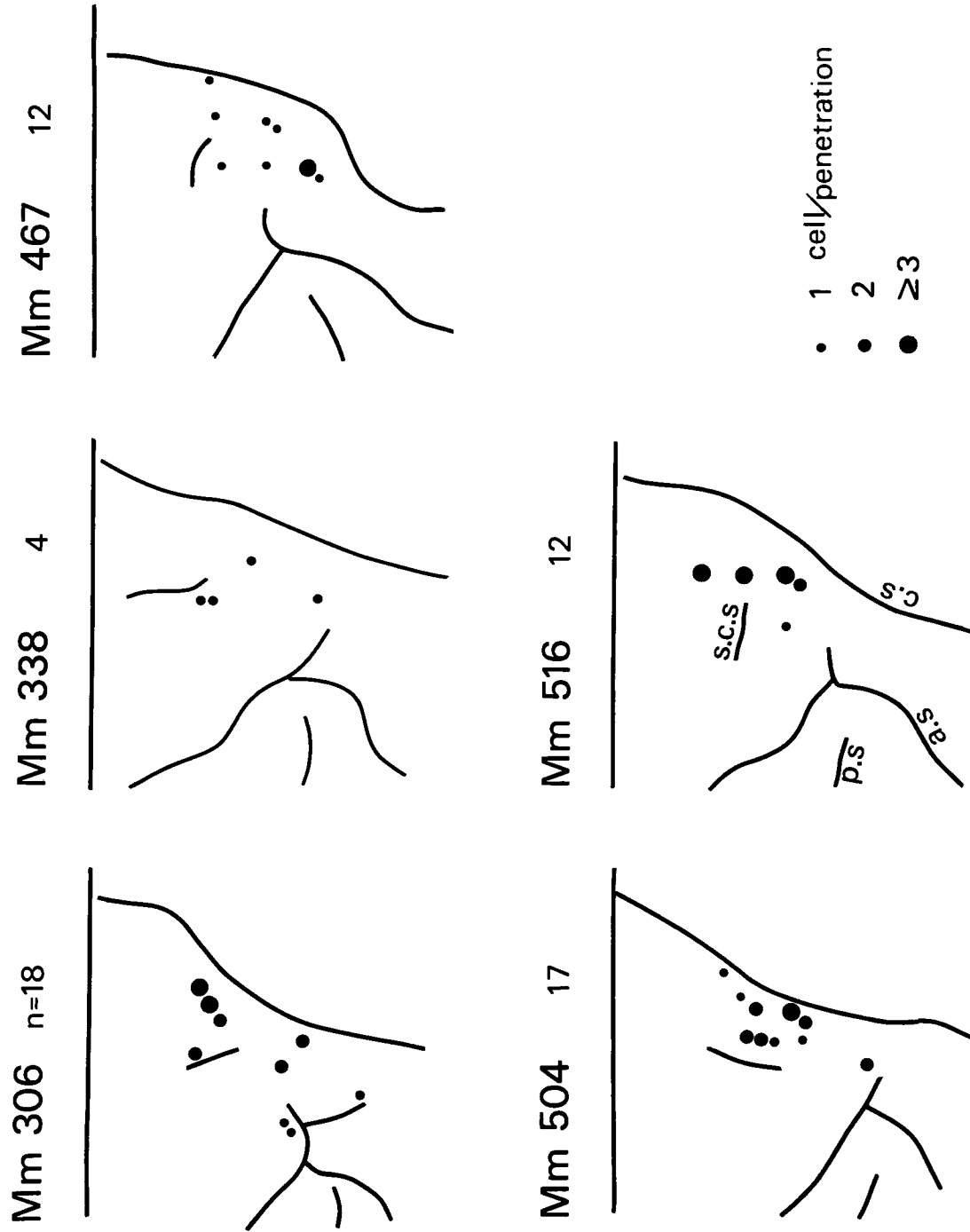


Fig.3

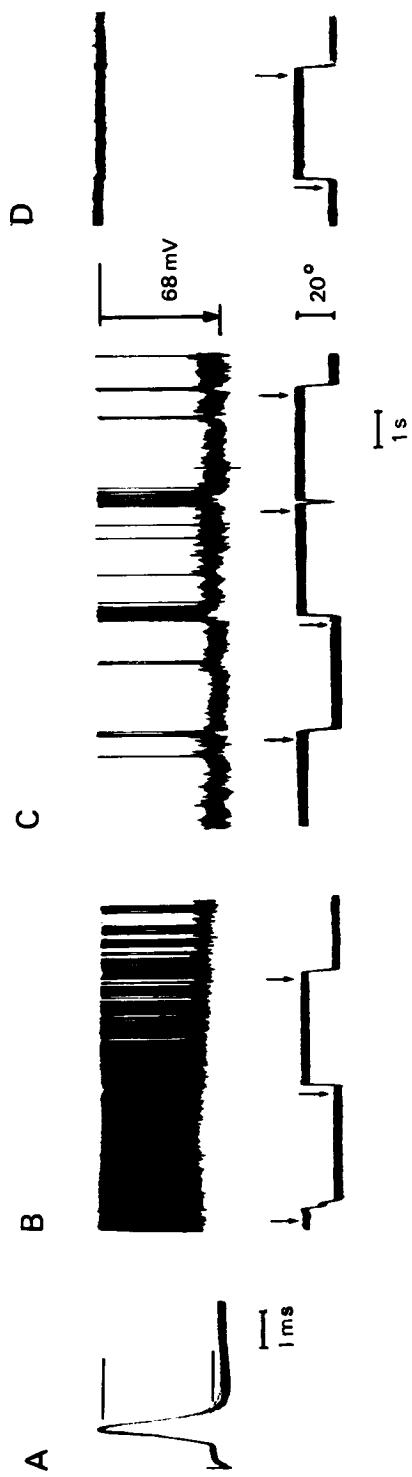


Fig.4

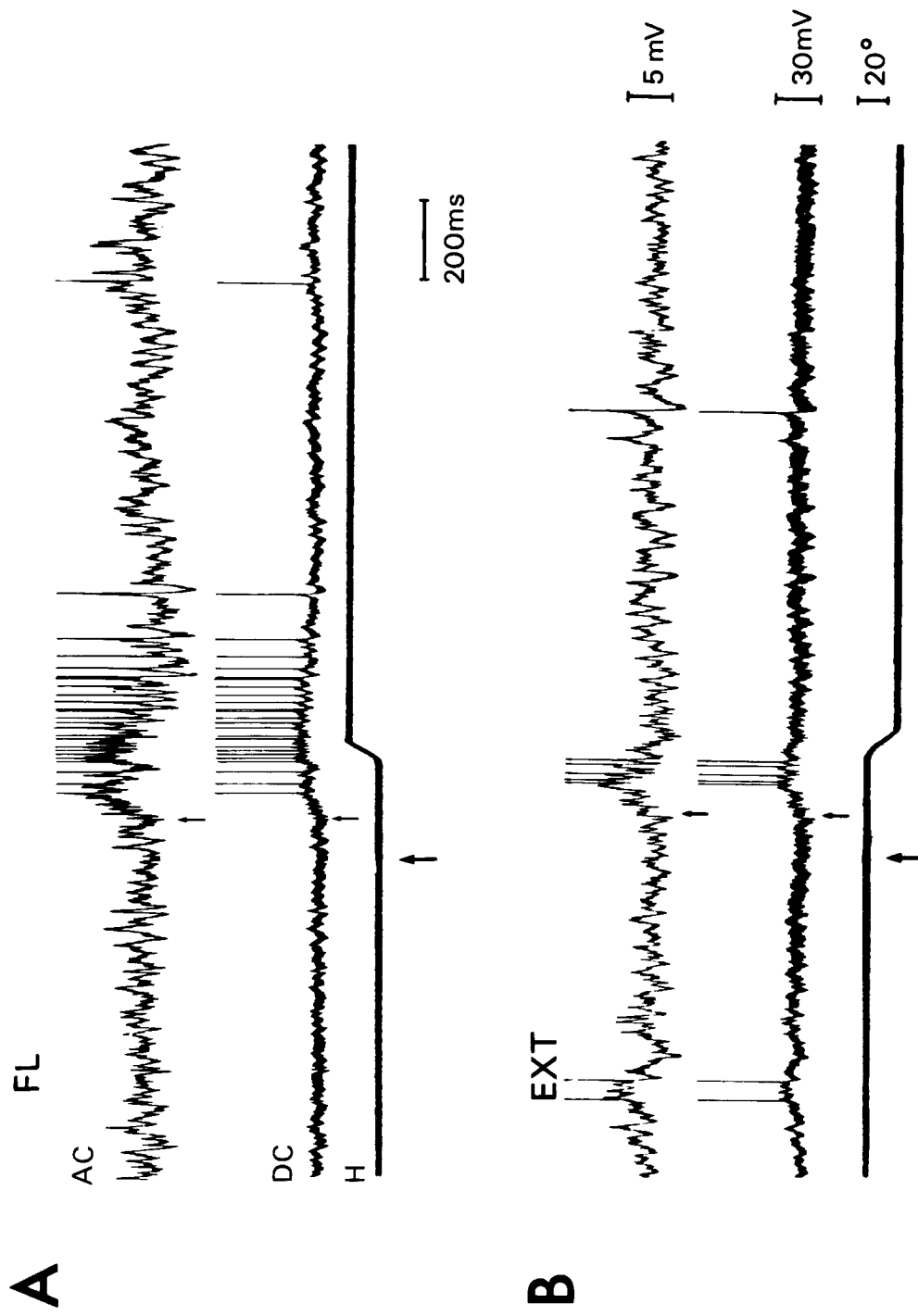


Fig.5

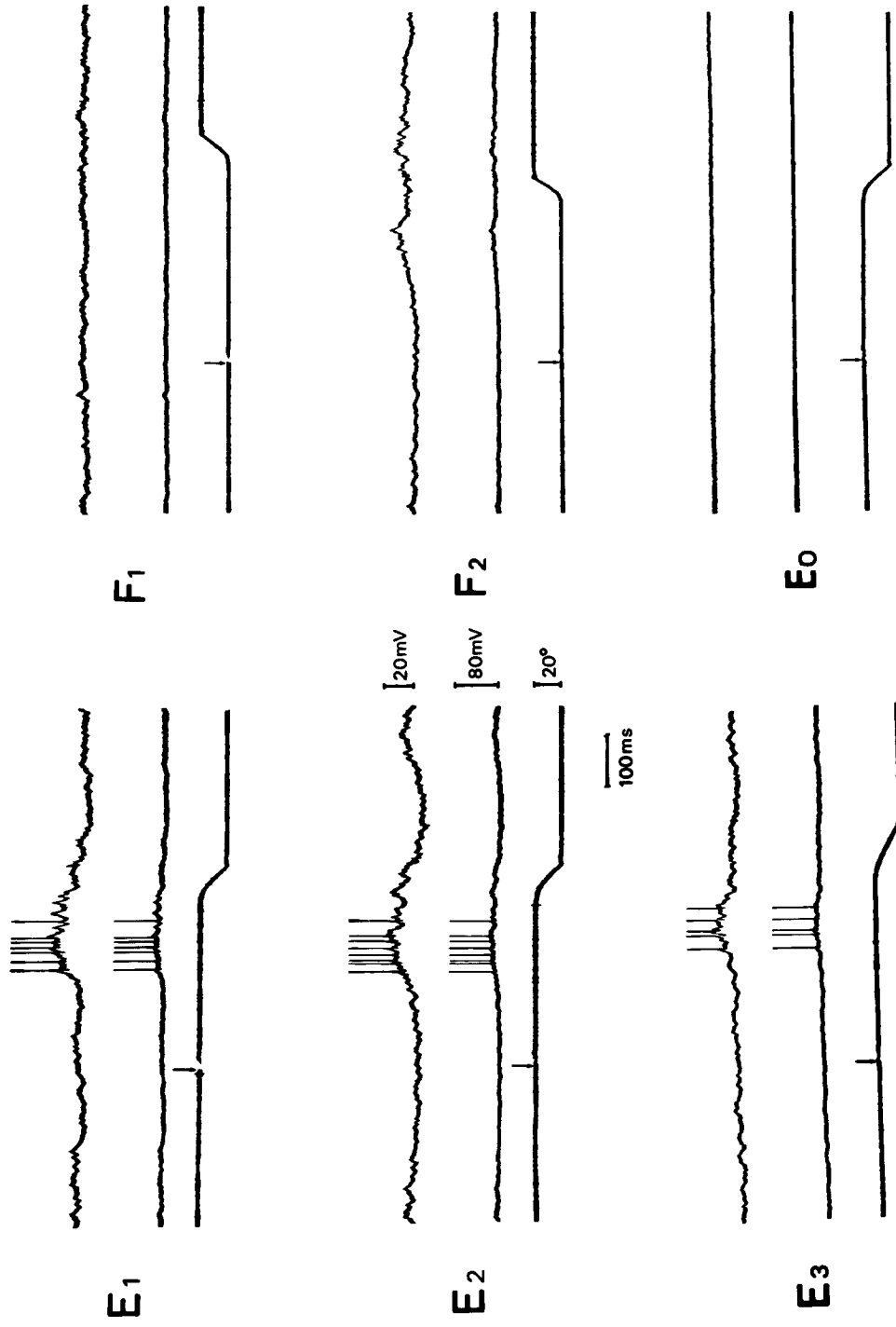


Fig.6

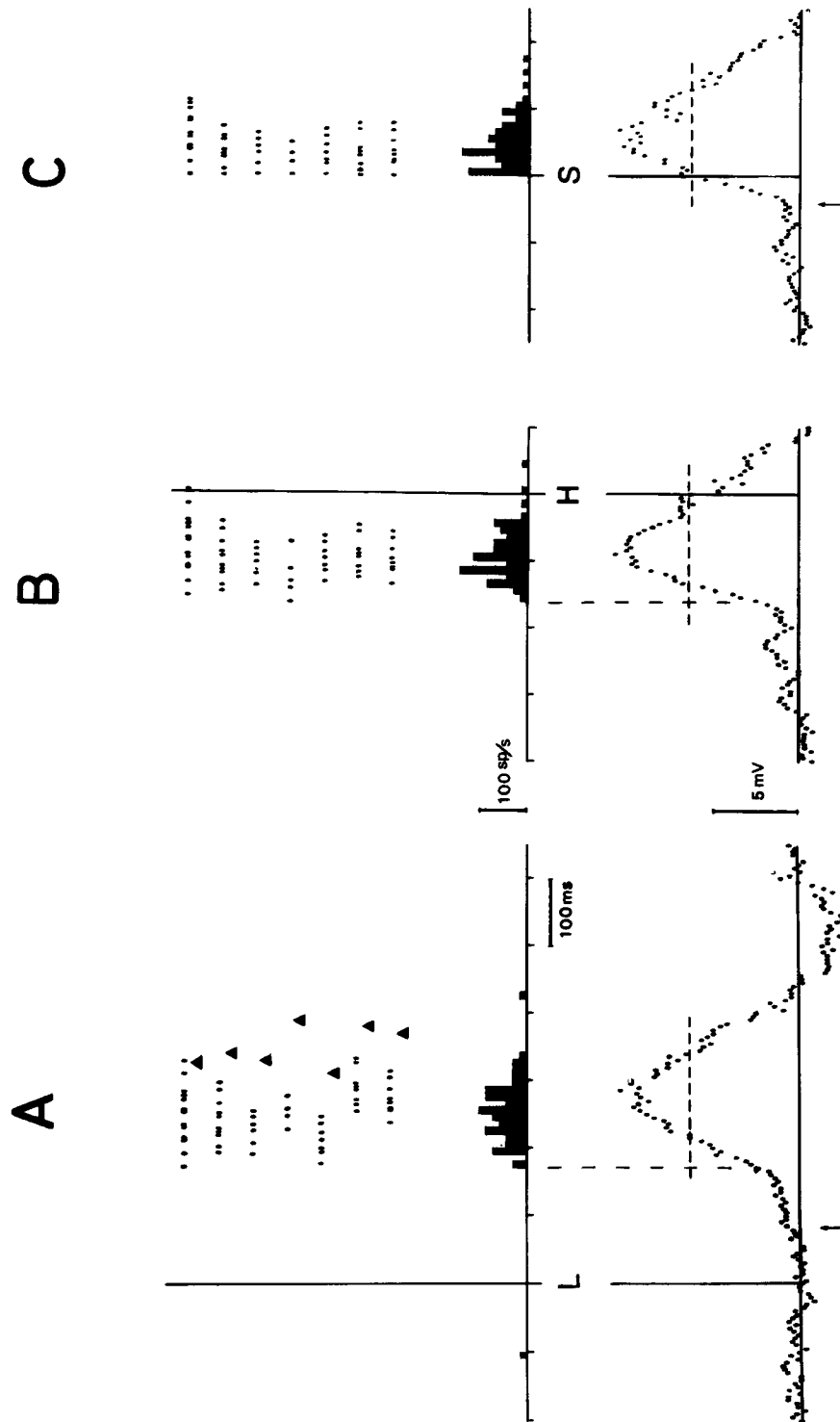




Fig.7

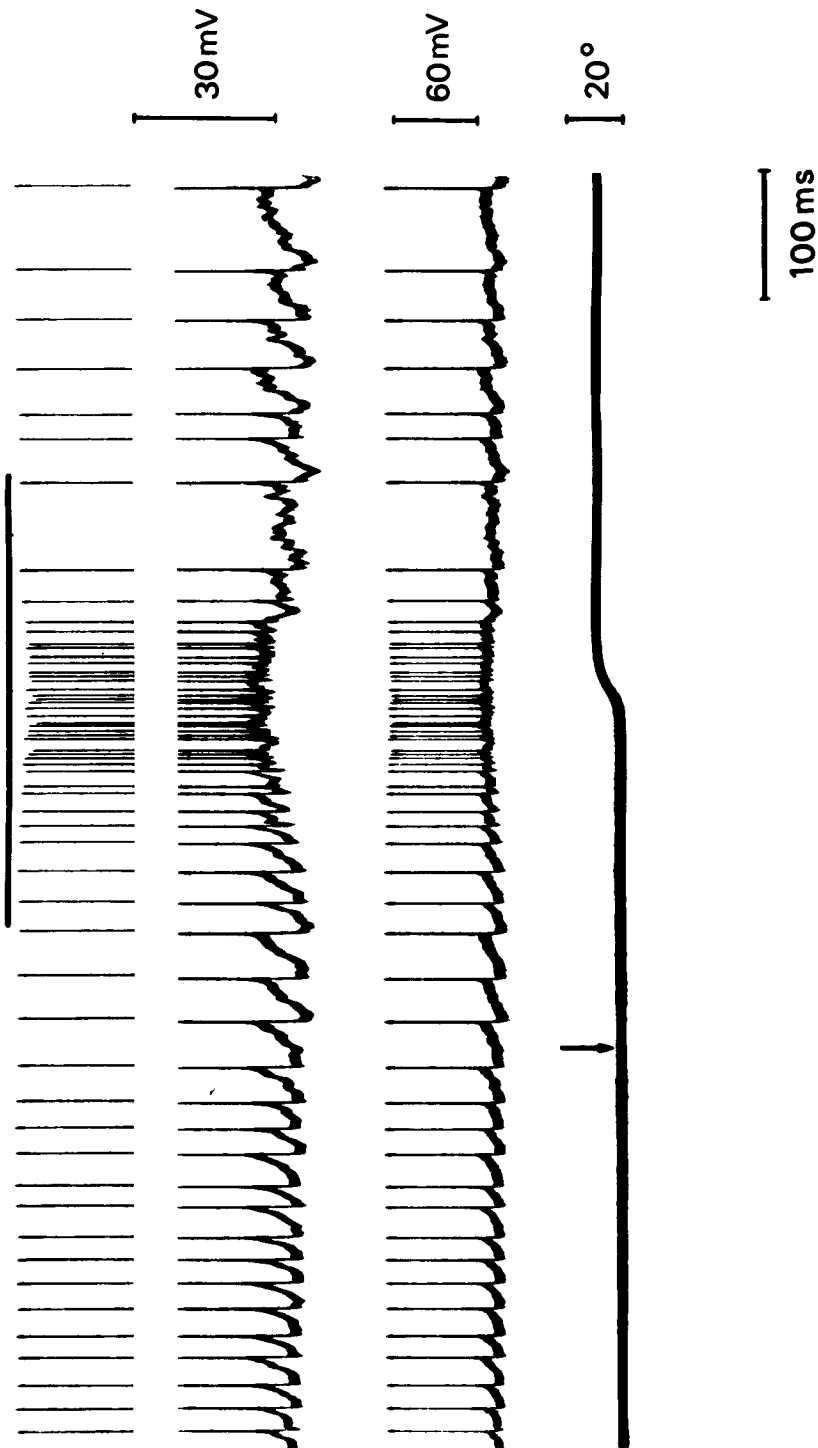


Fig.8

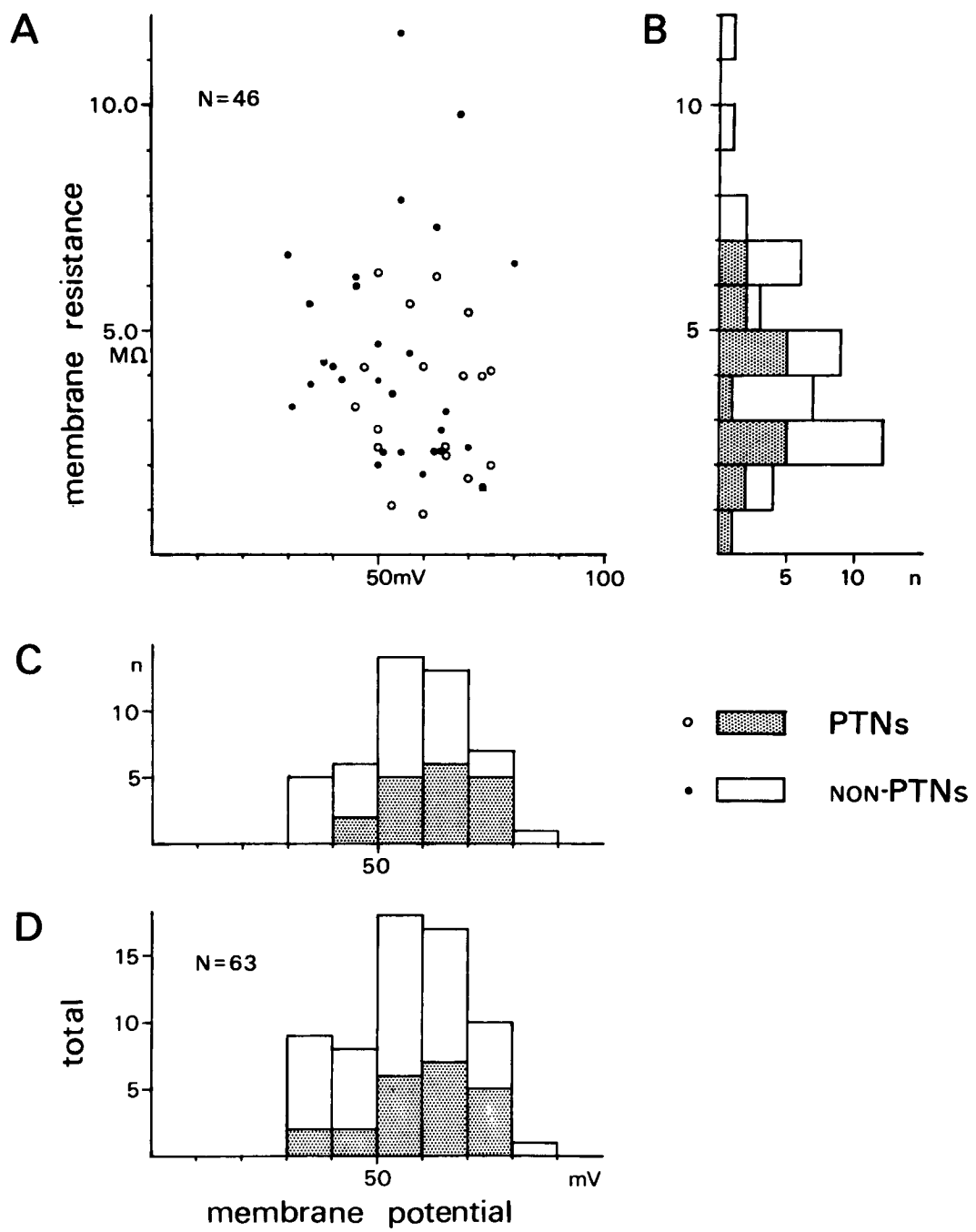


Fig.9

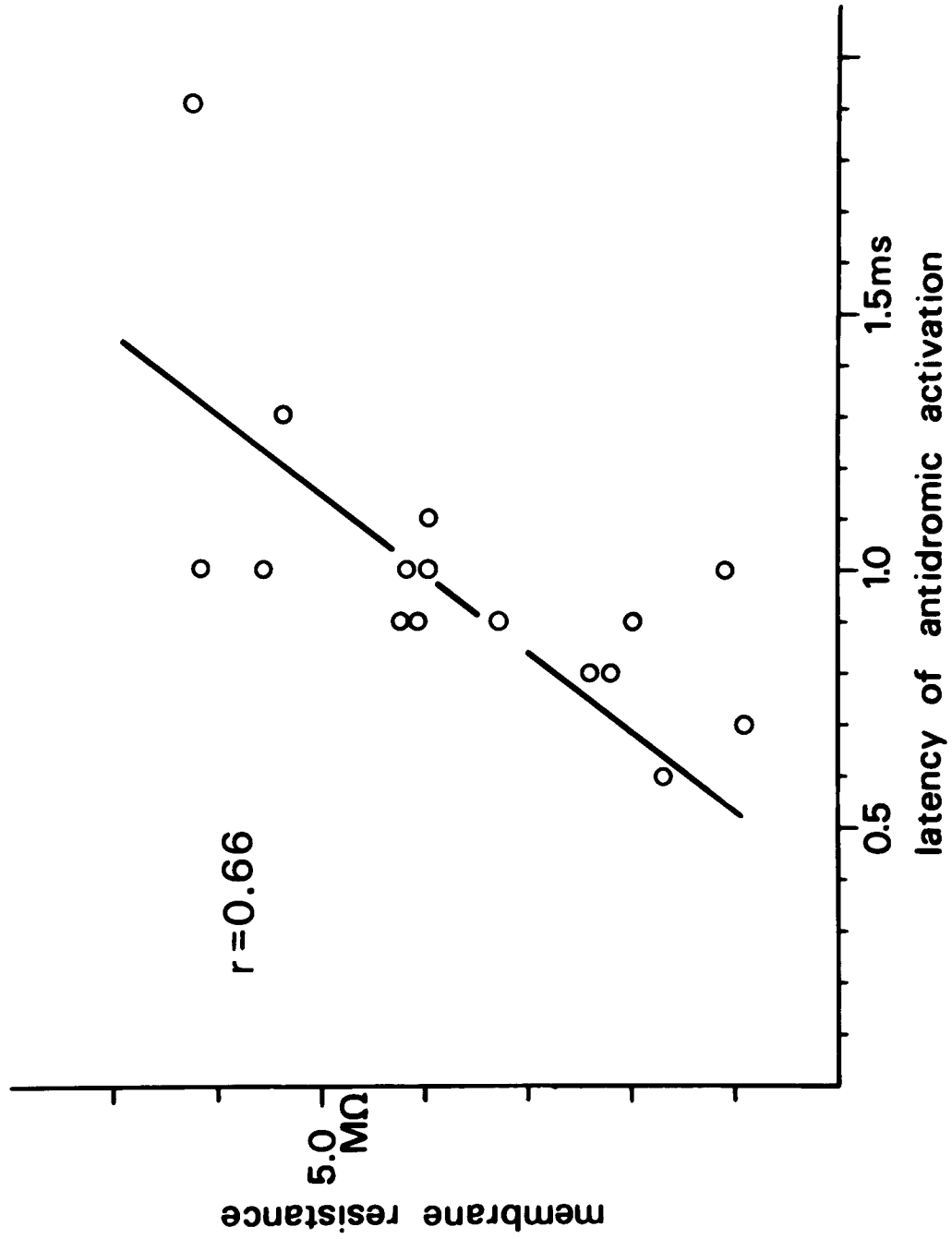


Fig.10

